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(84) Antigenically active proteins and peptides, and transmissible gastro-enteritis virus TGEV vaccines.

(57) The invention relates to new antigenic proteins which can be used to stimulate the immunity of pigs against transmissible gastro-enteritis virus (TGEV)-vaccines, and to fragments or derivatives thereof. The invention also relates to vaccines which comprise such antigenic proteins or peptides.

The nucleotide sequence and amine acid sequence of a gene coding for protein of an TGEV-strain are indicated in figure 1.

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New antigenically active proteins and peptides, and transmissible gastro-enteritis virus (TGEV)-vaccines.

The invention relates to a new antigenic protein which is capable of stimulating the immunity of pigs against transmissible gastro-enteritis virus (TGEV), and to fragments or derivatives thereof. The invention further relates to vaccines which comprises such an antigenic protein or peptide.

Transmissible gastro-enteritis is an infection disease of the intestine in pigs causing high mortality in piglings younger than 2 weeks and hence great economic losses to the pig breeders. In spite of the existence of various types of vaccines, the problem of TGE has not been solved effectively. Therefore, the development of new, better vaccines against TGE is urgently necessary.

The cause of TGE is a virus which belongs to the group of corona viruses. Corona viruses are positive single-stranded RNA viruses having a lipid envelope. The TGEV genome RNA has a length of approximately 23,600 bases. The virus particle (virion) comprises three important proteins, namely the peplomer protein (S), the nucleocapside protein (N), and the matrix protein (M).

The characteristic protrusions at the surface of the virion are formed by the peplomer protein (S). Each peplomer is formed by a glycopolypeptide. The protein is fixed in the membrane by means of a terminal membrane anchor sequence. It is known from literature that antibodies generated against the peplomer protein of the said virus, provide protection to the pig.

According to the present invention the nucleotide sequence and the amino acid sequence of the TGEV Purdue strain were determined.

The invention therefore relates to proteins and fragments thereof which comprise at least one of the areas which play a part in the protection against TGE infections.

Theoretically, such fragments can be used as such as an antigen for vaccinating against TGE. However, it is to be preferred to couple said fragments to a suitable carrier.

The peptide fragments or proteins according to the invention which comprise at least one antigenically active peptide fragment can be prepared according to methods known for the preparation of peptides and proteins.

First of all, the peptides can be prepared synthetically by means of known techniques starting from the individual amino acids or smaller peptide fragments.

The peptides can also be obtained biosynthetically using recombinant DNA techniques and expression systems, for example, by:

a) transformation of host cells with an expression vector which comprises a DNA, coding for an (the) antigenic determinant(s) (peptides in general);

b) expressing the genome inserted in the expression vector;

c) harvesting the cell culture, and

d) separating the synthesised peptide (protein).

The invention therefore also relates to a method of preparing a DNA molecule which codes for peptides according to the invention. Such a method comprises the following steps:

a) isolation of TGE single-stranded RNA;

b) synthesis of a cDNA strand complementary to the RNA strand mentioned in a); and

c) removal of the RNA molecule and synthesis of a second cDNA strand, using the first cDNA strand as a template.

The double-stranded DNA obtained in this manner may be incorporated in an expression vector in known manner so that a recombinant expression vector is formed. This vector may be introduced into a suitable host cell, for example, by transformation.

The invention will now be described in greater detail with reference to the ensuing specific example.

EXAMPLE

a) Virus and tissue culture.

TGEV strain Purdue was purified twice by means of pricking of a plaque on PD5 cells. Culture conditions were described previously in L. Jacobs et al, J. Virol. 57; (1986); 1010-15.

b) Isolation of subgenomic mRNA's

PD5 cells (9×10^7) were infected with an infection multiplicity of 15. Seven hours after infection actinomycin D was added and 10 hours after infection the cells were lysed and the RNA extracted, as described previously by W.J.M. Spaan et al., Virol. 108 (1981); 424-434. The RNA (560 μ g) was dissolved in a buffer having a high salt concentration (10 mM Tris-HCl pH 7.5, 0.05% SDS, 500 mM NaCl) and transferred to an oligo(dT)-cellulose column for a selection on poly(A) + RNA. After elution with 10 mM Tris-HCl pH 7.5 the RNA was concentrated by precipitation with ethanol and

fractionated by means of an isokinetic sucrose gradient (B.A.M. van der Zeijst et al., in G.D. Fasman (Ed.), Handbook of biochemistry and molecular biology, 3rd ed. Physical and Chemical data. Vol. 1 CRC Press Inc., Boca Raton, Fla., (1976), pp. 426-519). Before providing the RNA on the gradient it was dissolved in 50 mM Tris-HCl pH 7.4, 10 mM LiCl, 1% SDS and heated at 58°C for 1 minute. ³²P-labelled TGEV mRNA's were added as markers. The identification and translation of TGEV mRNA's is described by Jacobs et al., 1986.

c) Cloning of TGEV peplomer (E2) gene.

RNA3 obtained after purification over the sucrose gradient was used for the synthesis of cDNA (carried out as described by Gubler and Hoffman (Gene, 25 (1983), 263-269). Calf-thymus pentamers were used as primers and methyl mercury hydroxide for denaturing the RNA. The double-stranded cDNA was elongated with dC residues and then cloned in a pUC9 vector elongated with dG residues. The DNA was used for the transformation of *E. coli* strain JM109 as described by D. Hanahan et al., J. Mol. Biol., 166, (1983), 557-580. In this manner a cDNA library of approximately 900 transformants was obtained. The plasmides comprise insertions having a length up to 5kb.

d) Selection of E2-specific recombinants.

E2-specific recombinants could be identified by using the homology between TGEV and FIPV. Two restriction fragments from the E2 gene of FIPV were labelled with ³²P-dATP by means of nick translation and were used for the selection of the recombinants. Twenty-four recombinants hybridised with probe 2, eight with probe 1 and two recombinants hybridised with both probes. A restriction map of three recombinants was made. The recombinants which represent the whole peplomer gene were used for sequence analysis. Restriction fragments were separated in an agarose gel and isolated by means of an NA45 membrane (Schleicher and Schuell) and cloned in M13 vectors (mp8 and/or mp19). Sequence analysis was carried out according to the dideoxy-nucleotide termination method of Sanger et al., Proc. Natl. Acad. Sci. USA 74, (1977), 721-732. Both the universal M13 primer and E2-specific primers were used. The data were analysed by means of a DEC20/60 computer and the programs of Staden, Nucl. Acid. Res. 10, (1982), 4731-4751.

e) Nucleotide sequence.

The nucleotide sequence of the peplomer gene of TGEV (Purdue strain) is shown in figure 1. The sequence comprises an open reading frame of 4347 nucleotides with a coding capacity for a precursor protein of 1449 amino acids (nucleotide position 155-4502). Upstream of the ATG codon and downstream of first stop codon a recurring sequence (ACTAAACT) is present. This "intergenic" sequence also flanks the E2 gene of FIPV and has also been found upstream of the nucleocapsid gene of TGEV and MHV.

f) Amino acid sequence.

The ATG translation initiation codon (position 161) is succeeded by 15 amino acids which probably form the cleavable signal peptide, two hydrophilic amino acids succeeded by 13 hydrophobic amino acids. The extreme carboxy terminal part, position 4319-4393 (25 amino acids) comprises a hydrophobic region which presumably serves as a transmembrane anchor, both regions are underlined in Figure 1.

Claims

1. A gene which codes for protein of transmissible gastro-enteritis virus (TGEV), characterized by the nucleotide sequence as shown in Figure 1.
2. A protein, characterized by the amino acid sequence as shown in Figure 1, or a part thereof.
3. An immunogen, characterized in that it comprises a protein or peptide as claimed in Claim 2, whether or not coupled to a carrier.
4. A vaccine, characterized in that it comprises an immunogen as claimed in Claim 3.
5. A method of preparing a protein or peptide as claimed in Claim 2, characterized in that
 - a) the protein or peptide is synthesised in a manner known per se from individual amino acids and/or by coupling smaller peptides; or
 - b) a host cell is transformed with an expression vector which comprises a DNA coding for a protein or peptide as claimed in Claim 2, and the genome introduced in the expression vector is expressed.
6. A DNA molecule which comprises a nucleotide sequence which codes for a protein or peptide as claimed in Claim 2, or which codes for a polypeptide which comprises a protein or peptide as claimed in Claim 2.
7. A recombinant DNA expression vector which expresses the whole protein or peptide or a part thereof as defined in Claim 2, comprising an

operon with initiator sequences and terminator sequence and a nucleotide sequence which codes for the said protein or peptide, the said nucleotide sequence being situated between the initiator sequence and the terminator sequence of the operon.

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8. A host cell which comprises a recombinant DNA expression vector and/or DNA molecule as claimed in Claim 6 or 7.

9. A method of preparing a DNA molecule which codes for a protein or peptide as claimed in Claim 2, characterized in that

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a) single-stranded TGEV-RNA is isolated;

b) a cDNA strand complementary to said RNA strand is synthesised;

c) the RNA strand is removed and a second strand of cDNA is synthesised with the first cDNA as a template.

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* H T M K K L F V V L V V M P L I Y G D
 TTAACACACCATGAAAAAACTATTTGTGGTTTGGTTCGTAATGCCATIGATTTAIGGAGA
 160 170 180 190 200 210

N F P C S K L T N R T I G N Q W N L I E
 CAATTTTCCTTGTCTAAATTGACTAATAGAACTATAGGCAACCAAGTGGAAATCTCATTGA
 220 230 240 250 260 270

T F L L N Y S S R L P P N S D V V L G D
 AACCTTCCTTCTAAACTATAGTAGTAGGTTACCACCTAATTCAGATGTGGTGTAGGTGA
 280 290 300 310 320 330

Y F P T V Q P W F N C I R N N S N D L Y
 TTATTTTCCTACTGTACAACCTTGGTTTAATTGCATTGCAATAATAGTAATGACCTTTA
 340 350 360 370 380 390

V T L E N L K A V Y W D Y A T E N I T W
 TGTTACACTGGAAAATCTTAAAGCAGTGTATTGGGATTATGCTACAGAAAATATCACTTG
 400 410 420 430 440 450

N H R Q R L N V V V N G Y P Y S I T V T
 GAATCACAGACAACGGTTAAACGTAGTCGTTAATGGATACCCATACTCCATCACAGTTAC
 460 470 480 490 500 510

T T R N F N S A E G A I I C I C K G S P
 AACAACCCGCAATTTTAATTCTGCTGAAGGTGCTATTATATGCATTTGTAAGGGCTCACC
 520 530 540 550 560 570

P T T T T E S S L T C N W G S E C R L N
 ACCTACTACCACCACAGAATCTAGTTTGACTTGCAATTGGGGTAGTGAGTGCAGGTTAA
 580 590 600 610 620 630

H K F P I C P S N S E A N C G N M L Y G
 CCATAAGTTCCCTATATGTCCTTCTAATTCAGAGGCAAATTGTGGTAATATGCTGTATGG
 640 650 660 670 680 690

L Q W F A D E V V A Y L H G A S Y R I S
 CCTACAATGGTTTGCAGATGAGGTTGTTGCTTATTTACATGGTGCTAGTTACCGTATTAG
 700 710 720 730 740 750

F E N Q W S G T V T F G D M R A T T L E
 TTTTGAAAATCAATGGTCTGGCACTGTCACATTTGGTGATATGCGTGCGACAACATTAGA
 760 770 780 790 800 810

V A G T L V D L W W F N P U Y D V S Y Y
 AGTCGGTGGCAGCTTGTAGACCTTTGGTGGTTTAACTCTGTTTATGATGTCAGTTATTA
 820 830 840 850 860 870

R V N N K N G T T V U S N C T D Q C A S
 TAGGGTTAATAATAAAAAATGGTACTACCGTAGTTTCCAATTGCACTGATCAATGTGCTAG
 880 890 900 910 920 930

Y U A N U F T T Q P G G F I P S D F S F
 TTATGTGGCTAATGTTTTTACTACACAGCCAGGAGGTTTTATACCATCAGATTTTAGTTT
 940 950 960 970 980 990

N N W F L L T N S S T L V S G K L V T K
 TAATAATTGGTTCCTTCTAATACTAAGCTCCACGTTGGTTAGTGGTAAATTAGTTACCAA
 1000 1010 1020 1030 1040 1050

Q P L L U N C L W P V P S F E E A A S T
 ACAGCCGTTATTAGTTAATTGCTTATGGCCAGTCCCTAGCTTTGAAGAAGCAGCTTCTAC
 1060 1070 1080 1090 1100 1110

F C F E G A G F D Q C N G A U L N N T V
 ATTTTGTGTTTGAGGGGTGCTGGCTTTGATCAATGTAATGGTGCTGTTTTAAATAATACTGT
 1120 1130 1140 1150 1160 1170

D V I R F N L N F T T N U Q S G K G A T
 AGACGTCATTAGGTTCAACCTTAATTTTACTACAAATGTACAATCAGGTAAGGGTGCCAC
 1180 1190 1200 1210 1220 1230

V F S L N T T G G V T L E I S C Y T V S
 AGTGTGTTTCATTGAACACAACGGGTGGTGTCACTCTTGAAATTTTCATGTTATACAGTGAG
 1240 1250 1260 1270 1280 1290

D S S F F S Y G E I P F G U T D G P R Y
 TGACTCGAGCTTTTTTCAGTTACGGTGAAATTCGGTTCGGCGTAACTGATGGACCACGGTA
 1300 1310 1320 1330 1340 1350

C Y U H Y N G T A L K Y L G T L P P S V
 CTGTTACGTACACTATAATGGCACAGCTCTTAAGTATTTAGGAACATTACCACCTAGTGT
 1360 1370 1380 1390 1400 1410

K E I A I S K W G H F Y I N G Y N F F S
 CAAGGAGATTGCTATTAGTAAGTGGGGCCATTTTTATATTAATGGTTACAATTTCTTTAG
 1420 1430 1440 1450 1460 1470

T F P I D C I S F N L T T G D S D V F W
 CACATTTCTATTGATTGTATATCTTTTAATTTGACCACTGGTGATAGTGACGTTTTCTG
 1480 1490 1500 1510 1520 1530

T I A Y T S Y T E A L V Q V E N T A I T
 GACAATAGCTTACACATCGTACACTGAAGCATTAGTACAAGTTGAAAACACAGCTATTAC
 1540 1550 1560 1570 1580 1590

K V T Y C N S H V N N I K C S Q I T A N
 AAAGGTGACGTATTGTAATAGTCACGTTAATAACATTAAATGCTCTCAAATTACTGCTAA
 1600 1610 1620 1630 1640 1650

L N N G F Y P V S S S E U G L V N K S V
 TTTGAATAATGGATTTTATCTGTTTCTTCAAGTGAAGTTGGTCTTGTCATAAGAGTGT
 1660 1670 1680 1690 1700 1710

V L L P S F Y T H T I V N I T I G L G M
 TGTGTTACTACCTAGCTTTTACACACATACCATTGTTAACATAACTATTGGTCTTGGTAT
 1720 1730 1740 1750 1760 1770

K R S G Y G Q P I A S T L S N I T L P M
 GAAGCGTAGTGGTTATGGTCAACCCATAGCCTCAACATTAAAGTAACATCACACTACCAAT
 1780 1790 1800 1810 1820 1830

Q D H N T D V Y C I R S D Q F S V Y V H
 GCAGGATCAACACCGATGTGTACTGTATTCGTTCTGACCAATTTTCAGTTTATGTTCA
 1840 1850 1860 1870 1880 1890

S T C K S A L W D N I F K R N C T D V L
 TTCTACTTGCAAAAGTGCTTTATGGGACAATATTTTAAAGCGAAACTGCACGGACGTTTT
 1900 1910 1920 1930 1940 1950

D A T A V I K T G T C P F S F D K L N N
 AGATGCCACAGCTGTTATAAAACTGGTACTTGTCTTTCTCATTGATAAATTGAACAA
 1960 1970 1980 1990 2000 2010

Y L T F N K F C L S L S P V G A N C K F
 TTACTTAATTTTAAACAGTTCTGTTTGTGCTTGAGTCCTGTTGGTGCTAATTGTAAGTT
 2020 2030 2040 2050 2060 2070

D V A A R T R T N E Q V V R S L Y V I Y
 TGATGTAGCTGCCCGTACAAGAACCAATGAGCAGGTTGTTAGAAGTTTGTATGTAATATA
 2080 2090 2100 2110 2120 2130

E E G D N I V G V P S D N S G V H D L S
 TGAAGAAGGAGACAACATAGTGGGTGTACCGTCTGATAATAGTGGTGTGCACGATTTGTC
 2140 2150 2160 2170 2180 2190

V L H L D S C T D Y N I Y G R T G V G I
 AGTGCTACACCTAGATTCCTGCACAGATTACAATATATATGGTAGAACTGGTGTGGTAT
 2200 2210 2220 2230 2240 2250

I R Q T N R T L L S G L Y Y T S L S G D
 TATTAGACAACTAACAGGACGCTACTTAGTGGCTTATATTACACATCACTATCAGGTGA
 2260 2270 2280 2290 2300 2310

L L G F K N V S D G V I Y S V T P C D V
 TTTGTTAGGTTTTAAATGTTAGTGATGGTGTCTACTCTGTAACGCCATGTGATGT
 2320 2330 2340 2350 2360 2370

S A Q A A V I D G T I V G A I T S I N S
 AAGCGCACAAGCAGCTGTTATTGATGGTACCATAGTTGGGGCTATCACTTCCATTAAACAG
 2380 2390 2400 2410 2420 2430

E L L G L T H W T T T P N F Y Y Y S I Y
 TGAAGTGTAGGCTAACACATTGGACAACAACACTAATTTTATTACTACTCTATATA
 2440 2450 2460 2470 2480 2490

N Y T N D R T R G T A I D S N D F D C E
 TAATTACACAAATGATAGGACTCGTGGCACTGCAATTGACAGTAATGATTTTGTATTGTGA
 2500 2510 2520 2530 2540 2550

P V I T Y S N I G V C K N G A F V F I N
 ACCTGTCATAACCTATTCTAACATAGGTGTTTGTAAAAATGGTGCTTTTGTTTTATTAA
 2560 2570 2580 2590 2600 2610

V T H S D G D V Q P I S T G N V T I P T
 CGTCACACATTCTGATGGAGACGTGCAACCAATTAGCACTGGTAATGTCACGATACCTAC
 2620 2630 2640 2650 2660 2670

N F T I S V Q V E Y I Q V Y T T P V S I
 AAAGTTTACCATATCCGTGCAAGTGAATATATTCAGGTTTACACTACACCAAGTGTCAAT
 2680 2690 2700 2710 2720 2730

D C S R Y V C N G N P R C N K L L T Q Y
 AGACTGTTCAAGATATGTTTGTAAATGGTAACCCTAGGTGTAACAAATTGTTAACACAATA
 2740 2750 2760 2770 2780 2790

V S A C Q T I E Q A L A M G A R L E N M
 CGTTTCTGCATGTCAAACCTATTGAGCAAGCACTTGCAATGGGTGCCAGACTTGAAAACAT
 2800 2810 2820 2830 2840 2850

E V D S M L F V S E N A L K L A S V E A
 GGAGGTTGATTCCATGTTGTTTGTCTGAAAATGCCCTTAAATTAGCATCTGTTGAAGC
 2860 2870 2880 2890 2900 2910

F N S S E T L D P I Y K E W P N I G G S
 ATTCAATAGTTTCAGAACTTTAGACCCTATTTACAAAGAATGGCCTAATATAGGTGGTTC
 2920 2930 2940 2950 2960 2970

W L E G L K Y I L P S H N S K R K Y R S
 TTGGCTAGAAGGTCTAAAATACATACTTCCGTCCCATAAATAGCAAACGTAAGTATCGTTC
 2980 2990 3000 3010 3020 3030

A I E D L L F D K V V T S G L G T V D E
 AGCTATAGAGGACTTGCTTTTTTGATAAGGTTGTAACATCTGGTTTAGGTACAGTTGATGA
 3040 3050 3060 3070 3080 3090

D Y K R C T G G Y D I A D L V C A Q Y Y
 AGATTATAAACGTTGTACAGGTGGTTATGACATAGCTGACTTAGTATGTGCTCAATACTA
 3100 3110 3120 3130 3140 3150

N G I M V L P G V A N A D K M T M Y T A
 TAATGGCATCATGGTGCTACCTGGTGTGGCTAATGCTGACAAATGACTATGTACACAGC
 3160 3170 3180 3190 3200 3210

S L A G G I T L G A L G G G A V A I P F
 ATCCCTTGCAGGTGGTATAACATTAGGTGCACTTGGTGGAGGCGCCGTGGCTATACCTTT
 3220 3230 3240 3250 3260 3270

A V A V Q A R L N Y V A L Q T D V L N K
 TGCAGTAGCAGTTTCAGGCTAGACTTAATTATGTTGCTCTACAACTGATGTATTGAACAA
 3280 3290 3300 3310 3320 3330

N Q Q I L A S A F N Q A I G N I T Q S F
 AAACCAGCAGATTCTGGCTAGTGCTTTCAATCAAGCTATTGGTAACATTACACAGTCATT
 3340 3350 3360 3370 3380 3390

G K V N D A I H Q R S R G L A T V A K A
 TGGTAAGGTTAATGATGCTATACATCAAAGATCACGAGGTCTTGCTACTGTTGCTAAAGC
 3400 3410 3420 3430 3440 3450

L A K V Q D V V N I Q G Q A L S H L T V
 ATTGGCAAAAGTGCAAGATGTTGTCAACATACAAGGGCAAGCTTTAAGCCACCTAACAGT
 3460 3470 3480 3490 3500 3510

Q L Q N N F Q A I S S S I S D I Y N R L
 ACAATTGCAAAATAATTTCCAAGCCATTAGTAGTTCTATTAGTGACATTTACAATAGGCT
 3520 3530 3540 3550 3560 3570

D E L S A D A Q V D R L I T G R L T A L
 TGACGAATTGAGTGCTGATGCACAAGTTGACAGGCTGATCACAGGAAGACTTACAGCACT
 3580 3590 3600 3610 3620 3630

N A F V S Q T L T R Q A E V R A S R Q L
 TAATGCATTTGTGTCTCAGACTCTAACCAGACAAGCGGAGGTTAGGGCTAGTAGACAAC
 3640 3650 3660 3670 3680 3690

A K D K V N E C V R S Q S Q R R F G F C G
 TGCCAAAGACAAGGTTAATGAATGCGTTAGGTCTCAGTCTCAGAGATTTCGGATTCTGTGG
 3700 3710 3720 3730 3740 3750

N G T H L F S L A N A A P N G M I F F H
 TAATGGTACACATTTGTTTTCACTCGCAAATGCAGCACCAAATGGCATGATTTTCTTTCA
 3760 3770 3780 3790 3800 3810

T V L L P T A Y E T V T A W P G I C A S
 CACAGTGCTATTACCAACGGCTTATGAACTGTGACTGCTTGCCAGGTATTTGTGCTTC
 3820 3830 3840 3850 3860 3870

D G D R T F G L V V K D V Q L T L F R N
 AGATGGTGATCGCACTTTTGGACTTGTCGTTAAAGATGTCCAGTTGACTTTGTTTCGTAA
 3880 3890 3900 3910 3920 3930

L D D K F Y L T P R T M Y Q P R V A T S
 TCTAGATGACAAGTTCTATTTGACCCCAAGAACTATGTATCAGCCTAGAGTTGCAACTAG
 3940 3950 3960 3970 3980 3990

S D F V Q I E G C D V L F V N A T V S D
 TTCTGACTTTGTTCAAATTGAAGGGTGCGATGTGCTGTTTGTAAATGCAACTGTAAGTGA
 4000 4010 4020 4030 4040 4050

L P S I I P D Y I D I N Q T V Q D I L E
 TTTGCCTAGTATTATACCTGATTATATTGATATTAATCAGACTGTTCAAGACATATTAGA
 4060 4070 4080 4090 4100 4110

N F R P N W T V P E L T F D I F N A T Y
 AAATTTTAGACCAAATTGGACTGTACCTGAGTTGACATTTGACATTTTAAACGCAACCTA
 4120 4130 4140 4150 4160 4170

L N L T G E I D D L E F R S E K L H N T
 TTTAAACCTGACTGGTGAAATTGATGACTTAGAATTTAGGTCAGAAAAGCTACATAACAC
 4180 4190 4200 4210 4220 4230

T V E L A I L I D N I N N T L V N L E W
 CACTGTAGAAGTTGCCATTCTCATTGACAACATTAACAATACATTAGTCAATCTTGAATG
 4240 4250 4260 4270 4280 4290

L N R I E T Y V K W P W Y V W L L I G L
 GCTCAATAGAATTGAAACCTATGTAAATGGCCTTGGTATGTGTGGCTACTAATAGGCTT
 4300 4310 4320 4330 4340 4350

V V I F C I P L L L F C C C S T G C C G
 AGTAGTAATATTTTGCATACCATTACCTGCTATTTTGGCTGTTGTAGTACAGGTTGCTGTGG
 4360 4370 4380 4390 4400 4410

C I G C L G S C C H S I C S R R Q F E N
 ATGCATAGGTTGTTTAGGAAGTTGTTGTCACTCTATATGTAGTAGAAGACAATTTGAAA
 4420 4430 4440 4450 4460 4470

Y E P I E K V H V H * I * N V N S I I C
 TTACGAACCAATTGAAAAAGTGCACGTCCATTAAATTTAAAATGTTAATTCTATCATCTG
 4480 4490 4500 4510 4520 4530

Y N S S C F C * R I L L R M M N K V F K
 CTATAATAGCAGTTGTTTCTGCTAGAGAATTTTGTAAAGGATGATGAATAAAGTCTTTAA
 4540 4550 4560 4570 4580 4590

N * T
 GAACTAACT
 4600



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 88 20 0045

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
X	ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, vol. 185, no. 3, 1985, pages 63-82; S. HU et al.: "Studies of TGEV spike protein GP195 expressed in E. coli and by a TGE-vaccinia virus recombinant" * Whole article *	1-9	C 12 N 15/00 A 61 K 39/225 C 12 P 21/02 C 12 N 1/20
X,D	JOURNAL OF VIROLOGY, vol. 57, no. 3, March 1986, pages 1010-1015, American Society for Microbiology; L. JACOBS et al.: "Characterization and translation of transmissible gastroenteritis virus mRNAs" * Page 1014 *	2,3	
A	IDEM	1,5-9	
A	US-A-4 335 105 (P.M. GOUGH) * Whole document *	2-4	
X,P	THE JOURNAL OF GENERAL VIROLOGY, vol. 68, no. 7, July 1987, pages 1883-1890, SGM; D. RASSCHAERT et al.: "The predicted primary structure of the peplomer protein E2 of the porcine coronavirus transmissible gastroenteritis virus" * Whole article, especially figure 2 *	1-9	TECHNICAL FIELDS SEARCHED (Int. Cl. 4) C 12 N A 61 K C 12 P
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 14-04-1988	Examiner CUPIDO M.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			